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Cadaveric bone marrow as potential source of hematopoietic stem cells for transplantation

Jana Michalova,* Filipp Savvulidi, Ludek Sefc, Katarina Forgacova and Emanuel Necas
Center of Experimental Hematology and Institute of Pathological Physiology; First Faculty of Medicine; Charles University in Prague; Prague, Czech Republic

Every year, bone marrow transplantation saves many lives worldwide. Unfortunately, a suitable donor is not always available. Since organs are routinely harvested from cadaveric organ donors, we decided to assess such a possibility for bone marrow. We analyzed the functional properties and phenotypic markers of murine hematopoietic stem and progenitor cells (HSPC) from cadaveric bone marrow and during storage in vitro in a suspension. It was demonstrated that HSPC exposed to a warm or cold ischemia in intact femur did not lose their phenotype and maintained their repopulating ability for two to twelve hours depending on the temperature. Additionally, fresh bone marrow remained fully viable in cell suspension for two days or four days at 37°C or 4°C, respectively. Based on these findings, cadaveric bone marrow could be considered as an alternative source of hematopoietic stem cells for transplantation.

Key words: cadaveric bone marrow, mouse, transplantation, ischemia, hematopoietic stem cells

Abbreviations: BMT, bone marrow transplantation; CFU-S, colony-forming units-spleen; GM-CFC, granulocyte-macrophage colony-forming-cells; HLA, human leukocyte antigen; HSPC, hematopoietic stem and progenitor cells; LSK, $Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$; SLAM, $CD150^{+}CD48^{-}$

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*Correspondence to: Jana Michalova;
Email: jana.michalova@lf1.cuni.cz

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Allogenic bone marrow transplantation (BMT) is often the only hope for patients suffering from hematopoietic disorders, autoimmune diseases or solid tumors. For a successful BMT, one of the most important issues is the HLA-matching between the donor and host. If an HLA-matched donor is not found in the family, there is a chance of finding a donor from the registry of volunteers[1] or to use cord blood cells.[2] However, sometimes even these options are unsuccessful and the question arises of whether an additional way exists of obtaining hematopoietic stem cells, e.g. by harvesting bone marrow from cadaveric organ donors. Although organs for transplantation are usually obtained from donors after brain death with persistent circulation (heart-beating donors), harvesting after circulation arrest (from non-heart-beating donors) has also been widely studied over the last two decades.[3-5] From the late nineties, several studies have been published, which focused on the possibility of also harvesting bone marrow from cadaveric organ donors.[6-9] It was shown that cadaveric bone marrow cells can be stored up to seven days without an increase in apoptosis and that three days of storage does not affect the CD34-positive fraction of the cells.[7,9] Soderdahl et al.[9] assumed that these cells could be procured with a high degree of engraftment potential. This is not surprising when specific features of HSPC are considered. Hematopoietic stem cells reside in hypoxic regions of the bone marrow and are maintained in the G0-phase of the cell cycle for most of the time.[10,11] This is why they are called quiescent or dormant cells and as such may be assumed to be increasingly tolerant to unfavorable conditions arising post mortem and during storage.

In our study, we investigated the tolerance of murine HSPC to warm or cold ischemia and also to in vitro storage. Using Ly5.1/Ly5.2 congenic lines of mice in experimental bone marrow transplantations, we studied the phenotype, survival and function of HSPC collected at different times after the cessation of circulation or when maintained in a single-cell suspension.[12] Competitive repopulation assays, in both sublethally and lethally irradiated hosts, demonstrated that cadaveric HSPC engrafted normally when the bone marrow was left in situ for two hours, six hours and

twelve hours at 37°C, 20°C or 4°C, respectively. Similarly, they engrafted normally after two or four days of storage in a cell suspension at 37°C or 4°C, respectively.[12] Besides the crucial functional properties of HSPC tested by their transplantation, a higher resistance of HSPC compared to other bone marrow cells was confirmed via flow cytometry. The population of LSK ($Lin^-Sca\text{-}1^+cKit^+$) cells, enriched in HSPC, contained less apoptotic and dead cells than differentiated bone marrow cells after various periods of warm or cold ischemia employed in the experiments. Also the phenotype of HSPC, defined as LSK SLAM (LSK $CD150^+CD48^-$ cells),[13] was present as long as their transplantability was maintained.[12] Actively proliferating progenitors[14,15] tested as either CFU-S or GM-CFC, were more sensitive to ischemia or storage in suspension than the hematopoietic stem cells, which provide long-term donor chimerism. If cadaveric HSPC from the human bone marrow were also shown to be utilizable for transplantation, a new repository of bone marrow cells could be established. The bone marrow could be harvested in case of a sudden death, phenotyped and transplanted immediately or stored for later use.

Acknowledgments

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